

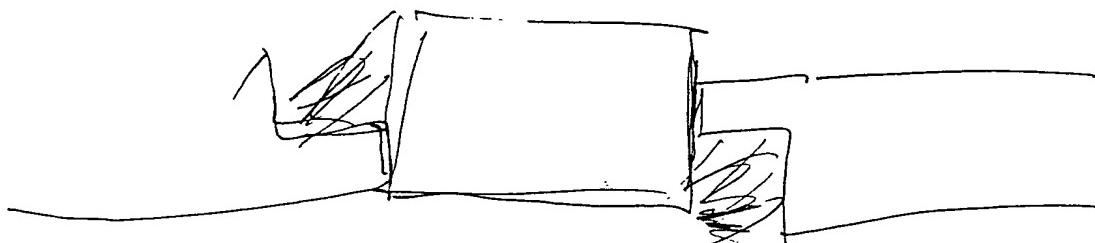
Reverse Complement DNA Sequence 09-YD - 19.Seq(1,263)

10 20 30 40 50 60
.....
TTTINGTTT TTACCTCGGG TINGAAATCG ATCGGGATAA AACTAACAAA ATCGGTATA 60
CGATAACGGT CGGTACGGGA TTTTCCCATC CTACTTCAT CGGGCTAC AAGGCTTCCC 120
AAGCTCACTC GGGAGCAACA GGATCTATTG TGGTGGAGTC GGGTGGGTC AGGTTATGAT 180
CGACCCGGTT ATTCTCCAT GGGTTTGIT GAGACTTCCT TCC 223

3' - cDNA 15 D₃

10 20 30 40 50 60

TTTCGTTT TTACCTCGGG TTCNAATCG ATCGGATAA AACTAACANA ATCGGTATA 60
CGATAACGGT CGGTACGGGA TTTTCCCATC CTACITTCAT CCCGGCTAC AAGGCTTCCC 120
AAGCTCACTC GGGAGCAACA GGATCTATTG TGGTGGAGTC GGGTGGGTC AGGTATGAT 180
CGACCCGGIT ATTCTCCAT GGGTTTGT TGAGACCTCC TCCACTACTC ATGAGCTCTC 240
TTCANT 246



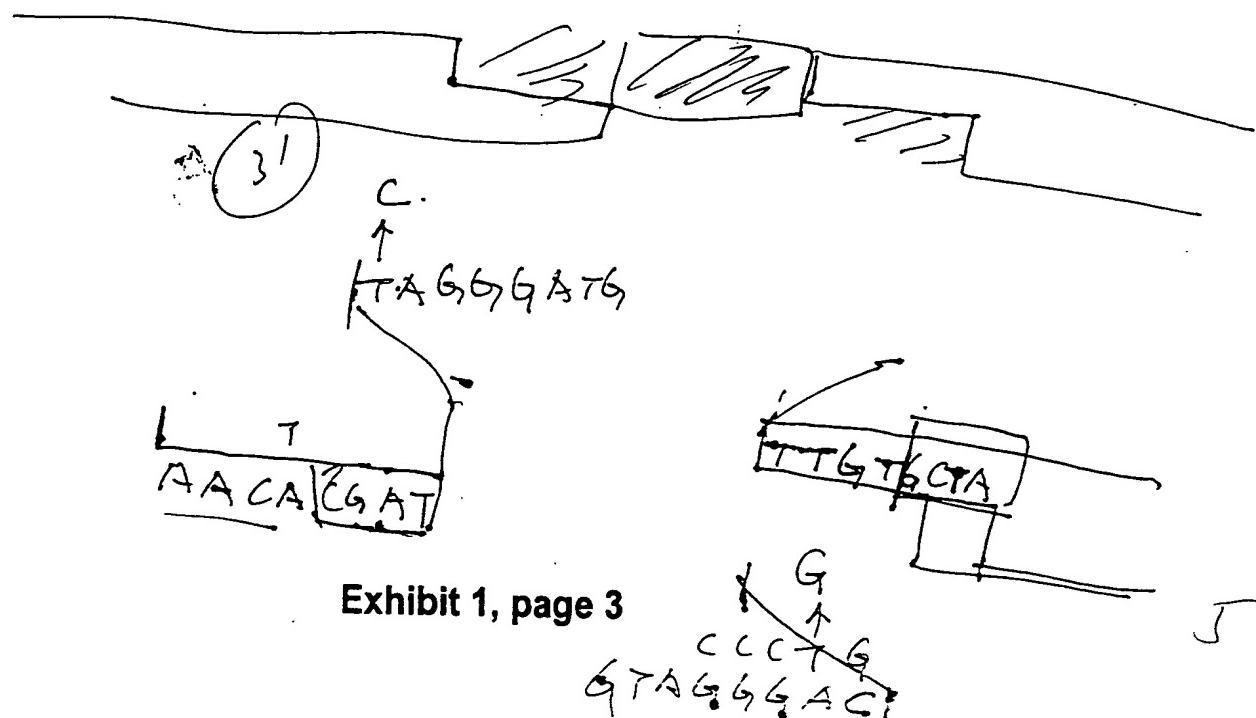
+ S

10 20 30 40 50 60

GTAGCATCGA TCTCTAACAA CGCTACCGT TTACCCGTAC CGGTAGACCC GGGTGITGIG 60
CTA~~AGGGAT~~ GAAAACGGTC GGTAACGGTC GGTAAAATAC CTCTACCGTT TTCATTTC 120
 TATTTAACCT GCAGGACGGA AACAAAACG GGATATAACCG GTAACNAAAA CGAACGGGAT 180
 AAATACGGTA ATCGAGTGNn nnnnnnnnn nnnnnnnnn nnnnnnnnTT TTCGITT 240
 ACCTCGGGIT CNAATCGAT CGGGATAAAA CTAACANAAT CGGTATACG ATAACGGTCG 300

310 320 330 340 350 360

TACGGGATT TTCCCATCCT ACTTTCATCC ~~CGGCTACAA~~ GGCTTCCCAA GCTCACTCGG 360
 GAGCAACAGG ATCTATTGIG GTGGAGTCGG GTGGGGTCAG GTTATGATCG ACCCGGTTAT 420
 TTCTCCATGG GGTTTGTGAG AGACCTCTTC CACTACTCAT GAGCTCTCTT CANT 474



Reverse Complement DNA Sequence 22-YWC - 282-33-2.Seq(1,699)

10 20 30 40 50 60
AAAGGAGAAA GAAAGAAGGA AAGGAAAANA GNAGGGGGGA AAGAAANGGN NANGAGNNA 60
GAAGANAGGA AGTGAAGGGG AGGGGAAGNG GAGAAAGGGG GAANGNTAA TINTTANGINA 120
GNGGTINNNA 120 ATTCTGTGAG AAACCCNGGT GATTTATGA GGGACACCGNG TGTTATNGTC 180
AATAGANNAN GAGAGATNCG GACAGAGACA CTGAAGAGIN NINGNINGGAA ACCAGGATCG 240
^AGACANGTC AACAGAGACN GNNGNAACCA ACGTTGAGAG GAATGGGINN AGCAGAGGTC 300
310 320 330 340 350 360
GANCGTCAGA GAATNGNAGN AGAAAAGAAG CAANTCACCN CCNOCACAGT CGGAGACACOG 360
TCATCAGTAN CNTNGATATC TAACCACCGTT ACCCGITNAC CGGTACCGGT AGACCCGGGT 420
GTITGTGCTAC AGGGATGAAA ACGNICTGGT ANC GGTINGGT TATATACTT TAACCTTGTT 480
TNGINITINA AAGINAACIN TGAGNGNCGT GAA 513

Large Scale production of Fleming Segm.

- use the original 1:50 dilution of 2nd round T-PCR product. ~~78~~ 78, 81, 33 and reagent of 36/2:50

78 (SGT 78)	DS5-3/AD3	12
36 (SGT 736)	DS3-3/AD3	12
81 (SGT 736)	DS5-3/AD3	24
33 (SGT 78)	DS3-3/AD3	24

cocktail:

DS3-3/AD3

A: for 33 and 36: 37 sets

DS5-3/AD3

B: for 78 and 81: 37 sets

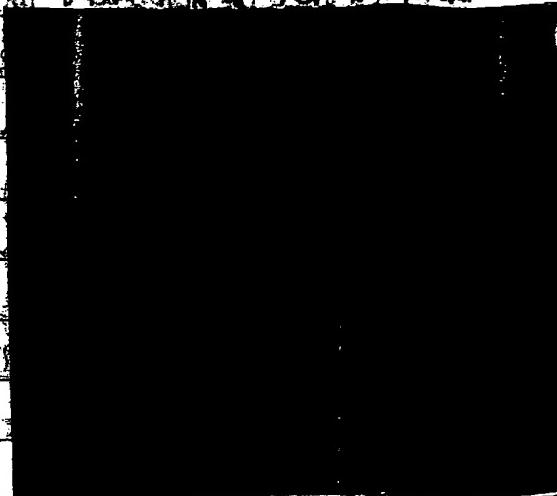
+ buffer	74	74
zgelz	59.2	59.2
tso	($\frac{1}{2}$) 2.9	432.9
dNTP	6.0	6.0
Ds primer	14.8	14.8
AD primer	74.0	74.0
Poly.	5.5	5.5
	2 3 2	
	6 6 6.4	6 6 6.4

33:?	444.2 M	81:?	444.2 M
NA:?	26.6 M	DA:?	26.6 M
36:?	222.1 M	78:?	222.1 M

Exhibit 2, page 1

Large scale production of 282/736 Flaming 3/5

Test run: 2% agarose gel 7/8/83



- 196 -

- purify the rest of 33. Ds3-3/8D3

§ 1: DST-3 | AD3

Recovered: - $18 \text{ cl} \times 21 = 378 \text{ cl}$,

added @ 6X loading cap. 73.6 = 451.6 lb.

- um 17% gel.

- cut out the bands. 133: 300 my x 3

mg: 30 mg x 5 (contaminated by 10%
bit 2 page 2 30% error by mistake)

Exhibit 2, page 2

✓ could be
contaminated by it
was thrown by mistake
using the bottle for
#3?

- store the gel block at 4°C

of N.Y.

Qiaquick - gel column - purify the fragment.

3 column / each sample. - multiple loadings

- twice wash

- elute in $50 \mu\text{l} \times 3 = 150 \mu\text{l}$ H₂O

- run off into cold in 2% agarose gel.

146 400 bp
2nd 372 (SGT736)
3rd 372 (SGT736)
4th 372 (SGT736)
5th 372 (SGT736)
6th 372 (SGT736)
7th 372 (SGT736)
8th 372 (SGT736)

smaller: SGT736 5' AD

larger SGT282 - 3' AD

stored: Box # FL-1 - 20°

- SGT736 5' AD3: *81-3 (Larger)

- SGT282 3' AD3: *33-3 (Large scale)

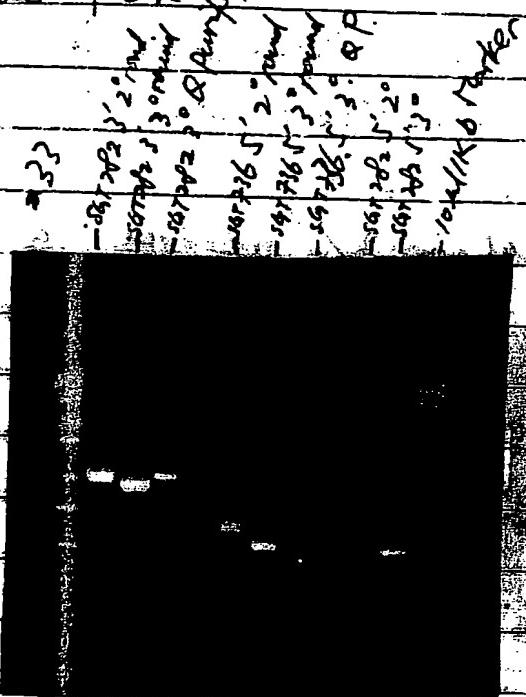
- run the second-round - Large-scale - product of
*33, *81 and *78

Larger

see the result on page 54.

Exhibit 2, page 3

The result of the 2nd-3rd round PCR of large scale PCR



~~L. Large~~ - 1.5 kb

- M: Median: 0.53Kb

S : small. : 0.3 Kb

3. 6. ~~5~~ set out of

3. 6. Cut out of 150 at larger scale
(see page 50-53).

*33 *81 *78

See page 46 for the background.

Notes:

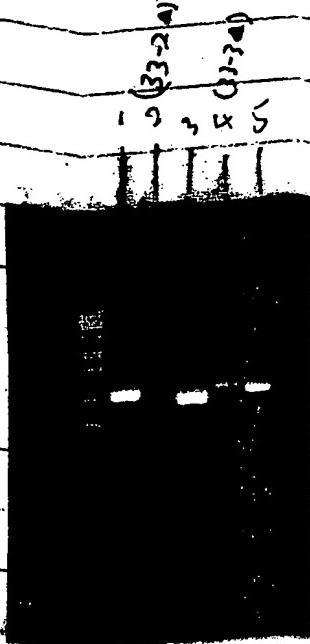
- The Q-purified bands ⁽³⁾ run slower (larger) than its original PCR product (2). Why? Cut out the ~1~3 bands purified by Q-F column to check it out (see the photo below for ~~example~~ the bands)
 - = #78 positive is a biggest good news: SGT2f2: 5' Flanking SO: The Flanking sequences from both ends have been already cut 3'-end Ds3 / AD3: cut 3 bands: L 1.5 kb, M 0.75 kb, S 0.3 kb
5'-end Ds5 / AD3: single band: ~500pb

Exhibit 2, page 4

Q-Q column purified the Cut-bands of #33-2, #33-3 and #78-3.

#78-3 (SGT282. 5'/AD2) was passed to York for cDNA screening

run the #33-2, #33-3 compared with the PCR original's



SGT282 DS5/AD3

1. original 3° PCR product. 10 μl

2. Q-purified 2° PCR product. 10 μl out of 50 μl

3. original 3° PCR product 10 μl

4. Q-purified 3° PCR Product 10 μl

5. 5 μl Q-purified 3° round Larger scale production

Conclusion OK.

stored: Box FL-1 -20°C

SGT282 3'-FL 2° round (2) : 33-2

SGT282 3'-FL 3° round (4) : 33-3

P-Cats: meeting on duty. taskin

SGT 282

- Run ~~the~~ purified the SGT282- 5' flake

- Cast out the 3 different size bait

- stored at 4°C

Exhibit 2, page 5

Q-Q purification onto in an 1.1% acrylamide gel

282-4AT3CRxx4AmfCR Map (1 > 1224) Site and Sequence
 Enzymes : All 206 enzymes (No Filter)
 Settings: Linear, Certain Sites Only, Standard Genetic Code

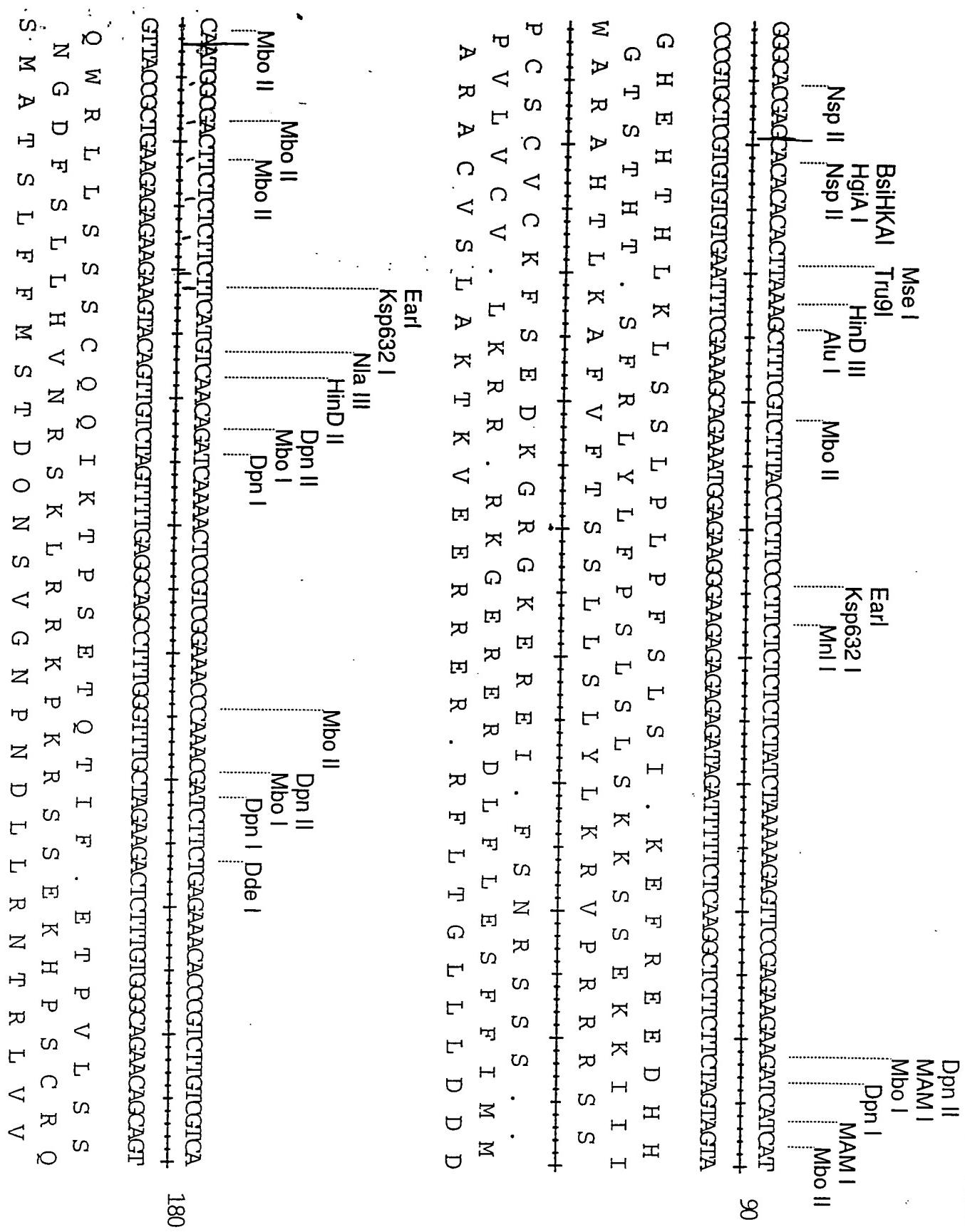


Exhibit 3, page 2

The diagram illustrates a DNA sequence and its analysis using various restriction enzymes. The sequence is:

V E R N G C S K A R A S S E N R R R K E A T R R R H S R R H V
T L R G M G V A K L F R L R I E E E K K Q L A A A T V G D T
T S L F P H L L A R A D S F R L L F S A V R R R W L R L C T
N L P I P T A F S S R R L I S S S F F C S A A A' V T P S V D

Below the sequence, a series of vertical lines represent restriction enzyme cleavage sites. The enzymes listed from top to bottom are:

- Csp6 I
- Rsa I
- Age I
- Bca77 I
- BsaWI
- Cfr10I
- PinAI
- Hpa II
- Acc I
- Csp6 I
- Hge II
- EcoP I
- Hpa II
- Nci I
- ScrF I
- Rsa I
- SfaN I
- Dpn I
- Mbo I
- Dpn I
- Taq I
- Cla I

A bracket labeled "Gloss" spans across the Hpa II site (indicated by a double-headed arrow). Below the sequence, a triangle points to the Hpa II site, with the label "Dsq." written vertically next to it.

At the bottom, two additional enzymes are shown: Alu I and Ava I.

Exhibit 3, page 3

S S V A S I S N N A T P C T R T G R P G V V L Q G S Q A H S
 . . Y C R D R V V S G R T G T G T S R T N H . L A R L S V R
 M L L M S R . C R . G T Y G Y R Y V P H Q A V L S E L E S P
 D T A D I E L L A V G H V R V P L G P T T S C P E . A . E P

Hinf I	Tfi I	Bca77I	BsaWl	Bcl I	Dpn II	Hpa II	Mbo I	Nla III	BsaJ I	Dsa I	Nla III
Bgl II	Dpn II	Mbo I	Xba II	Dpn I	BsaOI	Nci I	Sec I	BsaJ I	Dsa I	Alw26 I	
Dpn I					BsmA I						

GAGCACACAGCTATGCGCTAACGCTGGCTAACGATCATCACCGATTATCTCCATGGGTTCGAGCTCTAC
 CTCTGTTCTAGATAACCAACCTTACGGCCAGCCAGTCTACTAGTTCGGCTTAATAAGAGGTACCCAAACAACTCTGAGAGTG
 E Q Q D L L W W N P V G S S M I N P D Y S P W G L L R P L Y
 S N K I Y C G G I R S G Q V . S T R I I L H G V C . D L S T
 G A T R S I V V E S G R V K Y D Q P G L F S M G F V E T S L
 S C C S R N H H F G T P D L I I L G S . E G H P K N L G R .
 L L L I . Q P P I R D P . T H D V R I I R W P T Q Q S R E V
 A V L D I T T S D P R T L Y S . G P N N E M P N T S V E R S

540

~~Primer~~
~~40-3'~~

G1 = 23.

TACATGAGCTCTTGTATCAATCTAAATTGGTGCGATAATGCCGTCGACTNNNNNNNTACCOGGTTAATTT
ATGAGTACTCGGAGAACATAGAGTTAGGAGTTACACATGCCGAGCAGTTAGGACACTCTGAGNNNNNNNATGGCCATAAA

T H E L S S S I S I L K W C T L V Q . S L . D ? X X ? P G Y F
L L M S S L L , S Q S S N G V R S C N N R C E T X X X Y . P V I
· E H A R K . R L G . I T Y A R A I I A T L S X X X V R N N
V . S S E E I E I R L H H V S T C Y D S H S ? X X ? G P . K
S M L E R R D . D E F P T R E H L L R Q S V ? X X ? G T I K

Mbo II	Fin
Ava II	Dra II
PpuM I	Nla IV
Pss I	
Mbo II	
	Mse I
	Tru9I
Mae II	
Psp1406I	Hinf III
	Ssp I

AAAGTTACCCAAACCACCCCTGGAAAGGTTTAAGCTCAAAAAGTTAGAGTTAACATTTCGAAAAAGGTATAAG

720

Exhibit 3, page 5

F Q W V W W D L L P K F R S F F Q S Q I F K C . T F F P I F
 F N G F G G T F F Q N S G V F F N L K S S N V K R F F Q Y S
 F S M G L V G P S S K I Q E F F S I S N L Q M L N V F S N I
 K . H T Q H S R R G F N L L K K . D . I K L H . V N K G I N
 K L P N P P V K K W F E P T K K L R L D E F T L R K K W Y E
 E I P K T P G E E L I . . S N K E I E F R . I N F T K E L I R

Mae II
 Psp1406I
 Mbo II
 Bln I
 Dpn II
 Mbo I
 Dpn I Fok I
 Taq I
 Bln I
 Dpn II
 Mbo I
 Dpn I
 BSL I
 BsiY I
 Taq I
 Mbo II
 Mbo II
 HinF I
 Tfl I

GCTGCAACTCTCAAGAACGTTTGCATGGATCGATAATGTAGTTGATCCAAACGGTGGGATTTCGAACACACAATGA
 CGACCTGCAACGAAGTCTCTTGCACACTACCCTAGCTAGGTTGCCACCCCTAAAGCTTTTGTTGTA
 810

A G H L L Q E E T F G W D Q N N V V R S N G G D F R K T Q .
 L D T C F K K K R L D G I R I M . F D P T V G I F E K H N D
 R W T L A S R R N V W M G S E . C S S I Q R W G F S K N T M
 'A P C K S . S S V N P H S . F L T T R D L P P S K R F V C H
 S S V Q K L F F R K S P I L I I Y N S G V T P I K S F C L S
 Q V S A E L L F T Q I P D S V H L E I W R H P N E F F V I I

F F F R . T A T I S I F F N Q I I I R G A K V S F M I I E S
 S S S D E P L R S V F S S I R S S S E E P R F P L . S . N R
 I L L P M N R Y D Q Y F L Q S D H H Q R S Q G F L Y D H R I
 N K K R H V A V I L I K K L . I M M L P A L T E K I I M S D
 E E E S S G S R D T N E E I L D D D S S G L N G K H D Y F R
 R R G I F R . S . Y K R . D S . . L L W P K R . S . L I A
 +-----+-----+-----+-----+-----+-----+-----+-----+
 T T C T C T C T C O C G A T G A C C G T A C C A T C A G I T T T C T C A A T C A T C A T C A G A G G C C A A G G T T C C T T A T G A T C A T A G A T C G
 +-----+-----+-----+-----+-----+-----+-----+-----+
 A A C A A G A A G C T A C T T G G C G A T G C T A G I C T C C T C C A A A G G A A T A C T T G A T G I C T C C T C C G G T T C C A A A G G A A T A C T T G A C

rma | Spo | Mae | Fin |
 BspW | FnuH | rma | Ava II
 Ital | AlwN | Csp6 | Mnl I
 AuI | BbvI | RsaI | Nla IV
 ScaI | Tru9I | MnII |
 Spo |
 CTAGGAGCTTCAGTTCTGACTCTAGTACTACTATTAACTCTTAACTCAACGGGAAACAAATCATAACGGGACCAATGGG

066

Exhibit 3, page 7

L L K L Q K . Y . . D K N . R P L L D Y P V L P P I Q
 L A A E T E A E L V V I L G . K L S A V F . V P G I S S N P

BSL I
 BsiY I
 Bln I
 Mnl I
 Dpn II
 Mbo I
 Dpn I

Alu I
 Nla III
 Mae I
 rma I

Csp6 I
 Rsa I

BsaJ I
 BsaJ I
 Hpa II
 Sec I
 Nci I
 ScrF I

Hph I

G A T W K E T L E M D Q E V . R S T S F F R G N ' M V K E F Q
 E L H G R K P . K W I R R C E G V R V F S G E I W . K S F S
 S Y M E G N P R N G S G G V K E Y E F F P P G K Y G E R V S
 A V H F S V R S I S . S T H L L V L K K R P F I T F S N .
 S S C P L F G . F H I L L H S P T R T K E P S I H H F L K L
 L . M S P F G L F P D P P T F S Y S N K G P F Y P S L T E T

3GAGCTACATGGAAAGGAACCTAGAAATGGATCAGGAGGTACGAGTTTTCGGGAATAATGCTAAAGCTTCAG
 CCTCGATGTACCTTGGATCTTACCTAGTCCTCACACTCTCATGCTCAAAAAGCCCTTATAACACTTCTCAAGTC

HinD III
 Alu I
 Mse I
 Tru9I
 Dra I

Mae II
 Mae III
 Hph I

W W L Q R R H S . V I A V L I P L I C P . S F K C F I F L Y
 G G Y N V V T R R . L Q S . Y H . F V L E A L N V L S F Y I
 V A T T S S L V G D C S P N T I D L S L K L . M F Y L S I

1080
 1170

H H S C R R . E Y T I A T R I G N I Q G Q L K L H K I K R Y
P P . L T T V R L H N C D . Y W Q N T R S A K F T K D K . I
T A V V D D S T P S Q L G L V M S K D K F S . I N . R E I N

BsmA I
Mse I
Tru9I
Dra I
Esp3 I
Alw26 I Mse I
 Tru9I

IGATTAAACAAATCGTCCTTAAGAAAAACATTTAAGTAGATGAAGT
ACTAAATTGTTAGCACACAATTCTTTTGAAATTCACTACTTCA
1224

F K Q N R L F K E K T F . V D E S
D L N K I V S L K K K H F K . M K V
I . T K S S L . R K N I L S R . K
N L C F R R K L S F V N . T S S L
S K F L I T E K F F F C K L Y I F T
I . V F D D R . L F F M K L L H F H

itering library
1 colony \downarrow RF

OR

100 μ l \downarrow 5ml LB + 0.2% maltose + 10 μ M nysc \downarrow 0/n 37°C - OD₆₀₀ = 1.63 \downarrow 0.5ml \rightarrow 5ml LB + 0.2% maltose + 10 μ M nysc \downarrow 1.5 hr. OD₆₀₀ = 0.598 \downarrow

Spin down 4000 rpm 4 min

centrifugation

 \downarrow 600 μ l14 μ l \leftarrow 100 μ l \leftarrow 100 μ l \leftarrow

out phage library

(at) 14 μ l good

15°C 37°C

 \downarrow

Plating

37°C 8 hr.

Sequence	Primer	Primer	Primer	Primer
1 SGT26	80-26	D5-2	140-61	82
2 SGT26	80-3173	D5-3	140-61	82
3 SGT26	82-	D5-2	140-61	82
4 SGT26	82	D5-3	140-61	82
5 MB-5-1		T3 (10pm)	140-61	82
6 MB-5-1		T3	140-61	82
7 M7-1-2		T2	140-61	82
8 M7-1-2		T2	140-61	82

PCR #51 = 60 + 1

60, 95°C 75° - 1 min
 50°C 1 min 25X
 50°C 10 sec 100°C 10 sec

Screening for SCR282 cDNA

83

1° - 14 plate ($\approx 700,000$ phages) were screened with SCR282 probe (1 μ l from each PCR amplicon including both 3' end 5' flanking sequence) (1 μ l library - 1000 μ l \rightarrow 67 μ l/plate)

Washed 2xSSC, 1% SDS ~~for 65°C~~ 2 hr.

↓
exposed with intensifying screen, 24 hr.

5 positives \rightarrow 500 μ l SM + chloroform 4°C

SCR282-1, 282-2, 282-3, 282-4, 282-5

2° - positives were picked up and stored in 500 μ l SM + chloroform o/n.

dilution 1 μ l \rightarrow 1000 μ l SM

plating: 10 μ l, 20 μ l, 50 μ l/plate respectively
282-N., 1 2 3 (282 1-1, 1-2, 1-3)

Second Screening \times 65°C. hybridization, \times 2SSC, RT

↓ No.: 282, 1-1-1, 1-2-1

2-1-1 - -

3-1-1 -

4-1-1 -

5-1-1 - 5-1-1-2 -

Third Screening \times 65°C. H, \times 2SS Wash RT

No. 282. 3-1-1-1, ① ② . . .

$\frac{1}{3}$ (50-100) were positive 4-2-4, ② . ② - - from the same plates
5-1-1-1, ① ② . -

only 3, 4, 5 were positive at the third screen

No. 1, and 2 were missed

In Vivo excision

No. 2-1-1-1, ② 3-1-1-2 ① 3-1-1-2 ②

4-2-2-2 ① ②, 4-2-4-1 ② 6-2-4-1 ②

Exhibit 4, page 3

single phage were picked up and in VV0 excised our
 single colony was picked up and cultured in 20 µl C6
 + 5 µl of ml Amp 37°C, 10 hr.

DNA were isolated using QIAgene - kits 100.

dissolved in 20-50 µl water, to on much salt

Then diluted in to 300 µl and ethanol precipitate.

Dry in Speed Vac and dissolve DNA 100 µl water

EcoRI - XbaI cut.

5 µl DNA

1 µl EcoRI

1 µl XbaI

3 µl 10x UB buffer (Stratagene)

10 µl H2O

20 µl

37°C o/n.

G B A T D E F



Exhibit 4, page 4

Sequencing 282 catodase

No.	PCR tube	Sample	Primer
1	3111A	282-3111A	T ₃ → YWC 282-3T ₃ /T ₇
2		"	T ₇
3	4242A	282-42-4-2A	T ₃ YWC 282-4T ₃
4		"	T ₇
5		282-5121A	T ₃ YWC 282-5T ₃
6		"	T ₇ YWC 282-5T ₇
7	28	M3-1A	T ₃ YWC 3-1AT ₃
8		"	T ₇ YWC 3-1AT ₇
9		282-5121B	T ₃ YWC 282-5BT ₃
10		"	T ₇ YWC 282-5BT ₇

8 µl Terminator mix
 2 µl primers (20µM total)
 10 µl DNA
 2 µl

T₇ promoter does not work.

New sequencing is carried out as follows

PCR tube	Sample	Primer	mark for Nabel
1	282-3111A	T ₃	282-3AT ₃
2	282-3111A	T ₇ (from Xeromin)	282-3AT ₇
3	282-3111A	M13 Forward	282-3AMF
4	282-3111A	M13 Reverse	282-3AMR
5	282-4242A	T ₃	282-4AT ₃
6	"	T ₇	282-4AT ₇
7	"	M13 FP	282-4AMF
8	"	M13 RP	282-4AMR
9	282-4222A	T ₃	282-4BT ₃
10	"	T ₇	282-4BT ₇
11	"	M13 FP	282-4BMF
12	"		